

Perfluorooctanesulfonate and Other Fluorochemicals in the Serum of American Red Cross Adult Blood Donors

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Perfluorooctanesulfonyl fluoride-based products have included surfactants, paper and packaging treatments, and surface protectants (e.g., for carpet, upholstery, textile). Depending on the specific functional derivatization or degree of polymerization, such products may degrade or metabolize, to an undetermined degree, to perfluorooctanesulfonate (PFOS), a stable and persistent end product that has the potential to bioaccumulate. In this investigation, a total of 645 adult donor serum samples from six American Red Cross blood collection centers were analyzed for PFOS and six other fluorochemicals using HPLC-electrospray tandem mass spectrometry. PFOS concentrations ranged from the lower limit of quantitation of 4.1 ppb to 1656.0 ppb with a geometric mean of 34.9 ppb [95% confidence interval (CI), 33.3–36.5]. The geometric mean was higher among males (37.8 ppb; 95% CI, 35.5–40.3) than among females (31.3 ppb; 95% CI, 30.0–34.3). No substantial difference was observed with age. The estimate of the 95% tolerance limit of PFOS was 88.5 ppb (upper limit of 95% CI, 100.0 ppb). The measures of central tendency for the other fluorochemicals (*N*-ethyl perfluorooctanesulfonamidoacetate, *N*-methyl perfluorooctanesulfonamidoacetate, perfluorooctanesulfonamidoacetate, perfluorooctanesulfonamide, perfluorooctanoate, and perfluorohexanesulfonate) were approximately an order of magnitude lower than PFOS. Because serum PFOS concentrations correlate with cumulative human exposure, this information can be useful for risk characterization. **Key words:** American Red Cross, biomonitoring, blood donors, fluorochemicals, perfluorooctanesulfonate, perfluorooctanoate, PFOA, PFOS. *Environ Health Perspect* 111:1892–1901 (2003). doi:10.1289/ehp.6316 available via <http://dx.doi.org/> [Online 15 September 2003]

In May 2000, the 3M Company (3M) announced that it would voluntarily cease manufacturing materials based on perfluorooctanesulfonyl fluoride (POSF; C₈F₁₇SO₂F) after a metabolite of this compound, perfluorooctanesulfonate (PFOS; C₈F₁₇SO₃⁻), was found to be widespread in human populations and wildlife (3M Company 2003; Giesy and Kannan 2001; Hansen et al. 2001; Kannan et al. 2001a, 2001b, 2002a, 2002b). Using POSF as a basic building block, unique chemicals were created by further reactions with functionalized hydrocarbon molecules. Major applications of these POSF-based products have included surfactants, paper and packaging treatments, and surface protectants (e.g., for carpet, upholstery, textile). Depending on the specific functional derivatization or the degree of polymerization, such POSF-based products may degrade or metabolize, to an undetermined degree, to PFOS, a stable and persistent end product that has the potential to bioaccumulate. Although not a major commercial product, PFOS has been used in some products, including fire-fighting foams.

The mechanisms and pathways leading to the presence of PFOS in human blood are not well characterized but likely involve environmental exposure to PFOS or to precursor

molecules and residual levels of PFOS or PFOS precursors in industrial and commercial products. PFOS has been detected at low parts per billion (nanogram per milliliter) concentrations in the general population (3M Company 2003; Hansen et al. 2001; Olsen et al. 2003b), although the scope and sample size of these investigations have been limited. Serum PFOS concentrations among production employees working in POSF-related processes have averaged between 0.5 and 2 ppm depending on work activity (range < 0.1–12 ppm) (Olsen et al. 1999, 2003a, 2003c). A large body of toxicology and epidemiology data is available for review regarding PFOS [3M Company 2003; Organisation for Economic Co-operation and Development (OECD) 2002]. Results from several repeat-dose toxicologic studies consistently demonstrated that the liver is the primary target organ (3M Company 2003; OECD 2002). Liver cell hypertrophy and reduction in serum cholesterol are early responses to PFOS that occur in rats as well as monkeys. Both species display an apparent threshold for the toxic effects of PFOS that can be expressed in terms of cumulative dose or body burden, with no observable response at lower cumulative doses or body burdens. Following the company's announcement to

voluntarily cease production, the U.S. Environmental Protection Agency (EPA) finalized a significant new use rule to regulate PFOS and related chemicals (U.S. EPA 2002).

The purpose of this study was to better characterize the distribution of PFOS and six other fluorochemicals, some of which are PFOS precursors, in a large adult population by analyzing serum samples obtained from donors at six American Red Cross blood banks. An assessment of the serum fluorochemical distribution was performed in relation to three demographic attributes (age, sex, and location) of the anonymous blood donors.

Materials and Methods

Fluorochemicals

The seven analytes detected and quantified in this study were PFOS, *N*-ethyl perfluorooctanesulfonamidoacetate [PFOSAA; C₈F₁₇SO₂N(CH₂CH₃)CH₂COO⁻], *N*-methyl perfluorooctanesulfonamidoacetate [M570; C₈F₁₇SO₂N(CH₃)CH₂COO⁻], perfluorooctanesulfonamidoacetate (M556; C₈F₁₇SO₂NHCH₂COO⁻), perfluorooctanesulfonamide (PFOSA; C₈F₁₇SO₂NH₂), perfluorooctanoate (PFOA; C₇F₁₅COO⁻), and perfluorohexanesulfonate (PFHS; C₆F₁₃SO₃⁻).

PFOSAA is an oxidation product of *N*-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE), which was primarily used in paper and packaging protectant applications. M570 is an oxidation product of *N*-methyl

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The laboratory analysis of the seven fluorochemicals was provided by a dedicated team at Tandem Labs (formerly Northwest Bioanalytical; Salt Lake City, UT, USA). Individuals included A. Hoffman, C. Sakashita, P. Bennett, R. Foltz, S. Newman, T. Peacock, and E. Yardimici. We also thank J. Butenhoff and K. Young for their contributions.

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perfluorooctanesulfonamidoethanol, which was used primarily in surface treatment applications (e.g., carpets, textiles). Therefore, PFOSAA and M570 can be considered markers of consumer-related exposure. Both PFOSAA and M570 can metabolize to M556 and PFOSA, which in turn can later metabolize to PFOS. Unlike PFOSAA and M570, other POSF-related analytes (M556, PFOSA, and PFOS) are not specific to any one consumer application. PFOA and PFHS are not precursors or metabolites of PFOS. Ninety-seven percent of PFOA that was produced by 3M was used by its industrial customers and in its own processes as a fluoropolymer processing aid (Wendling 2003). The remainder was used in medical film coating and electronic applications involving printed circuit boards and precision bearings. 3M analytical studies of eight POSF-based product samples have identified the presence of PFOA at very low concentrations as a manufacturing impurity (Wendling 2003). In a biodegradation study of N-EtFOSE, PFOA could be formed instead of PFOS only under an abiotic or hydrolytic condition during the last step of the degradation pathway (Lange 2001). PFOA could also be an oxidation product or metabolite of the widely used telomer-based fluorochemicals manufactured by other companies (Hagen et al. 1981). PFHS, the sulfonate form of perfluorohexanesulfonyl fluoride (PHSF), could also be a residual by-product of POSF-based production. 3M also produced PHSF as a building block for compounds incorporated in fire-fighting foams and specific postmarket carpet treatment applications.

We also calculated the total organic fluorine value (TOF). TOF is the percentage of the molecular weight for each of the seven fluorochemicals that was attributed to organic fluorine (PFOS, 64.7%; PFHS, 61.9%; PFOA, 69.0%; PFOSAA, 55.3%; PFOSA, 64.7%; M570, 56.6%; M556, 58.1%) multiplied by the concentration measured for each fluorochemical and then summed across all seven fluorochemicals.

Sample Collection

Through cooperation with six American Red Cross blood banks, 645 serum samples (332 males, 313 females) were obtained from adult donors (20–69 years of age) in 2000–2001. Each sample (1–2 mL serum) was stored at –20°C in a plastic tube until laboratory analysis. Methodologic studies have not shown these fluorochemicals to be extracted from blood collection materials (Hansen K. Personal communication). The six American Red Cross blood banks represented donors from the following areas: Los Angeles, California; Portland, Oregon; Minneapolis–St. Paul, Minnesota; Charlotte, North Carolina; Hagerstown, Maryland; and Boston, Massachusetts. These

metropolitan areas were chosen to represent different geographical locations in the United States. (The primary 3M POSF-based production facility in the United States was located in Decatur, AL, which is not in the area of any of these six locations. 3M did manufacture PFOA at its Cottage Grove, MN, plant, which is located in the southeastern part of the Minneapolis–St. Paul metropolitan area. At this site there was also some production of POSF-based materials but not to the same extent as manufactured at the Decatur plant.)

Approval for this study was obtained from the American Red Cross Biomedical Services Institutional Review Board. Samples were void of personal identifiers. The only known demographic factors were age, gender, and location. Each blood bank center was requested to provide approximately 100 serum samples: 10 samples for each 10-year age interval (20–29, 30–39, 40–49, 50–59, and 60–69) for each sex. We estimated *a priori* a statistical power of more than 95% to detect a 20% difference between any two 10-year age groups (combined locations) and more than 80% power to detect a 20% difference by sex within each age group.

Laboratory Assay

Tandem Labs (formerly Northwest Bioanalytical; Salt Lake City, UT) analyzed the fluorochemicals using techniques similar to those described by Hansen et al. (2001). Some modifications were made to the method to accommodate the small sample volume and to further assure the quality of the method for the analysis of human sera and plasma samples (Tandem Labs 1999, 2001a, 2001b).

Sample preparation. One hundred microliters of serum (or plasma) were extracted for analysis. The serum sample was added to 400 μ L of 50 mM ammonium acetate in water in a polypropylene tube. Samples were vortex mixed and 50 μ L of the internal standard, tetrahydroperfluorooctanesulfonate (THPFOS), was added to each sample before a second vortex mixing of approximately 30 sec. Three milliliters of methyl-*tert*-butyl ether (MTBE) was then added to each sample, which was vortex mixed for 30 sec and rotated for 10 min. The samples were centrifuged, and the MTBE layer was transferred to a clean polypropylene tube. The MTBE was evaporated to dryness, and the extract was reconstituted in 100 μ L 30:70 (vol:vol) 20 mM ammonium acetate in water:20 mM ammonium acetate in methanol and vortex mixed for 15 sec. The samples were transferred to autovials and centrifuged at 3,000 rpm for 2 min.

Because of the difficulty in finding suitable human sera in the United States to use for method blanks and quality control (QC) samples, we obtained rural Chinese human plasma, collected in 1999, with low endogenous

fluorochemical concentrations, from a source within the United States. Studies designed to characterize the selectivity and the extraction efficiency for the two matrices, conducted before the sample analysis, indicated that this plasma was a suitable choice for the calibration, blank, and QC matrix samples.

Blank samples were prepared in the same way as the sera samples. The internal standard solution was added to half of the blank plasma samples; the remaining plasma blanks were prepared without internal standard. The THPFOS internal standard was added to all plasma and serum samples at approximately 200 ppb. THPFOS is a partially fluorinated surfactant with a structure similar to the target analytes and is a reasonable surrogate for all the analytes in this study. Studies carried out before validation indicated that, because THPFOS has a retention time very similar to PFOA, extremely high levels of PFOA (> 500 ppb) can suppress the signal for THPFOS, resulting in artificially high reported levels of all analytes except PFOA. However, none of the samples analyzed in this study contained PFOA at high enough levels to suppress the THPFOS signal.

Calibration standards and QC samples, extracted from plasma, were prepared following the same procedure described for the samples except that the QC standards were spiked with a mixture of the seven target analytes concurrent with the addition of the internal standard.

Given the low-level presence of many of the target analytes in these biologic matrices, rigorous attention to the preparation, analysis, and data interpretation of blanks, calibration standards, and QC samples was critical. The analytical system was monitored for analytical artifacts such as carryover and for potential sources of contamination.

Analysis of samples. We used a Genesis Lightning C18 4- μ m inner diameter, 2 \times 50 mm analytical column (Argonaut Technologies, Inc., Foster City, CA) for chromatographic separation. The mobile phase was 20 mM ammonium acetate in water (a) and 20 mM ammonium acetate in methanol (b) and was programmed to run from 50% to 97.5% (b) over the course of a 9-min gradient. After a 2-min hold at 97.5% (b), the run equilibrated back to 50% (b) for 2 min. The flow rate was 300 μ L/min and the column was maintained at 40°C. 2-(2-Methylethoxy)-ethanol (50 μ L/min) was added postcolumn.

Extracts were analyzed on a Perkin-Elmer Sciex API3000 with a TurboIon Spray source (PE Sciex, Concord, Ontario, Canada) maintained at 400°C in the negative ion mode. All seven target analytes, along with the internal standard, were monitored in a single run recording a single transition for each ion with a dwell time of 70 msec. The collision energy was optimized for each analyte and ranged

from 25 to 80 eV. Because the focus of this work was quantitation of the total amounts of the target analytes, isomers of the target analytes were treated as a single peak even though full resolution was achieved. Quantitation was based on evaluation of the samples versus duplicate calibration curves extracted from plasma predetermined to contain little or no endogenous levels of the target analytes. The calibration curves were evaluated using a quadratic regression analysis (weighted $1/X^2$).

Method characterization and QC. Before analysis of the samples, we validated this method with respect to selectivity, accuracy, precision, quantitation range, and lower limit of quantitation (LLOQ). The extraction efficiency of the target analytes from human serum was between 15% and 70%, depending on the analyte. It was not necessary to correct for extraction efficiency because the calibration curve was extracted. The precision and accuracy of the method were determined for all analytes by analyzing three levels of QC samples in replicates of five in a single run. Intra-assay precision was within 6% for all analytes; the accuracy was $\pm 18\%$ for all analytes.

The LLOQ, evaluated for each run and analyte, varied between 1 and 4 ppb. We did not perform an experimental determination of the method LLOQ; instead, the lowest standard injected for a particular run that met the acceptance criteria was chosen as the LLOQ. Samples were evaluated quantitatively using a 6–8-point extracted calibration curve covering the target range for each analyte from about 1 to 500 ppb. For all analytes except PFOSA and PFOSAA, at least 75% of the back-calculated concentrations for the calibration standard was required to be within 15% (20% at LLOQ) of the theoretical concentration. For PFOSA and PFOSAA, at least 75% of the back-calculated concentrations for the calibration standard was required to be within 20% (25% at LLOQ) of the theoretical concentration.

We found that QC samples, calibration standards, and samples were stable through seven freeze–thaw cycles. All analytes were stable in matrix through 42 days at -20°C . All analytes were stable in the extracts through 7 days stored at room temperature.

Analytical QCs, extracted from plasma and prepared at the same time as the calibration curve, were injected intermittently during the analytical run. Three levels of QC samples, spanning the range of the method (4, 150, and 400 ppb), were analyzed in duplicate. Analytical QCs were analyzed in duplicate; dilution QCs were also prepared and analyzed and run in triplicate for any assay that contained a diluted sample. QC samples were prepared at three levels spanning the calibration range: approximately 4, 150, and 400 ppb. The measured concentration for two-thirds of all analytical QCs was required to be within

20% (25% for PFOSA and PFOSAA) of the theoretical concentration, and no two QCs at the same concentration could be outside the limit. Any analytical run containing a diluted sample included a dilution QC, analyzed in triplicate for each dilution level. At least two of the dilution QCs were required to be within 20% of the theoretical concentration. Evaluation of QC samples injected during the analytical runs of the 645 samples indicated that the reported quantitative results may have varied $\pm 10\%$ for precision and accuracy (Tandem Labs 2002).

Twenty-four samples were split and analyzed to provide an estimate of the reliability of the analyses conducted. The analytical laboratory was blind to the identity of these split samples. These analyses were performed concurrently with all other analyses of the study to minimize experimental error. Five split samples were analyzed from Charlotte, Los Angeles, Hagerstown, and Portland and four split samples from Boston. Inadvertently, no reliability analyses were performed on the Minnesota samples. There was a strong correlation between the split samples ($r = 0.9$) with PFOS, PFOA, and PFHS. The analysis of the split samples for the other fluorochemicals was problematic because only six split samples for PFOSAA and seven split samples for M570 had values that were above the LLOQ. None of the PFOSA and M556 split samples were above the LLOQ.

Data Analysis

We used measures of central tendency applicable to log-normally distributed data (median, geometric mean) for descriptive analyses. In those instances where a sample was measured below the LLOQ, the midpoint between zero and the LLOQ was used for calculation of the geometric mean. A sensitivity analysis of the assessment of this midpoint assumption and how it affected the calculation of the geometric mean was performed using the 10th and then 90th percentile values between zero and the LLOQ for those samples $< \text{LLOQ}$. Results for the geometric mean calculations for each fluorochemical remained similar to when the midpoint assumption was used (data not shown).

The log-linear relation between PFOS and PFOA was modeled as follows:

$$\ln[\text{PFOS}] = (a \times \ln[\text{PFOA}]) + (c \times \text{age}) + (d \times \text{sex}) + (f \times \text{age} \times \text{sex}) + g + \varepsilon, \quad [1]$$

where g is the intercept and ε is the error term. We used an analogous log-linear model to relate PFOS to PFHS. The interaction term between age and sex was not a significant predictor in either model and was therefore not included in the final analyses.

Residuals were inspected to assure model assumptions provided reasonable fit.

To examine the relationship between PFOS concentration and the concentration of the two other precursor molecules, PFOSAA and M570, a nonlinear model was fit to the data:

$$\ln[\text{PFOS}] = \ln([\text{PFOSAA}]^a + [\text{M570}]^b) + (c \times \text{age}) + (d \times \text{sex}) + (f \times \text{age} \times \text{sex}) + g + \varepsilon. \quad [2]$$

This model represents an additive relation between PFOS concentration and the concentrations of the other two molecules because the hypothesized mechanisms of association—that is either correlated exposure sources or conversion from one molecule to another—suggested additivity rather than a multiplicative relation. At the same time, the model is consistent with the simpler models relating PFOS concentration to that of a single molecule and preserves the log-linear relationship of PFOS concentration to age and sex suggested by inspection of the residuals in these simpler models. The adjusted log-linear models were fit using maximum likelihood using the *lm* program, and the adjusted nonlinear model was fit using weighted nonlinear least squares, as implemented in the *nls* program; both are programs in S-Plus, version 6.0 (Insightful Corporation 2001). To avoid making normality assumptions in these log-linear models, bootstrapping was used to form confidence intervals for the parameters (Efron and Tibshirani 1993). In this method, a large number of full-size samples of the original observations are drawn with replacement, from each of which an estimate of the percentile is generated. The distribution of these estimates mimics the underlying sampling distribution for the original estimate assuming that the parent population looks like the sample. We used bias-corrected, accelerated percentiles to minimize residual bias. A bias correction factor is derived by comparing empirical percentiles with bootstrap percentiles, and acceleration is accomplished by partial jackknifing, a method of systematically resampling the data. The effect on parameter estimates and confidence intervals of including location as a random effect in the above models (using the S-Plus programs *lme* and *nlme*) was negligible; only the results from the nonhierarchical models are given. In order to minimize parametric assumptions in the estimation of extreme percentiles of the population, the bias-corrected, accelerated bootstrap method was also used to generate confidence intervals around the empirical percentiles for serum concentrations.

Results

The frequency distributions of PFOS, PFOA, PFHS, PFOSAA, and M570 are shown in Figure 1. Although the graphs suggest

log-normal distributions, only the PFOS distribution met such criteria based on the Shapiro-Wilk test. This lack of log normality is due to the greater percentage of subjects with values < LLOQ for PFOA, PFHS, PFOSAA, and M570. Statistical analyses are not presented for PFOSA and M556 because of the low number of subjects ($n = 13$) whose serum concentrations of PFOSA or M556 exceeded the LLOQ. The LLOQs for PFOSA and M556 ranged between 1.0 and 3.2 ppb. Although PFOSA and M556 are not presented

in the later analyses, they were included in the calculation of the TOF value assuming the midpoint between zero and the LLOQ.

The range, interquartile range (IQR; i.e., the lower end of the second quartile and the upper end of the third quartile), number of samples < LLOQ, 90th percentile, median, geometric mean, and 95% confidence interval (CI) of the geometric mean for PFOS, PFOA, PFHS, PFOSAA, and M570 are provided in Table 1. The midpoint assumption was based on the LLOQs in place for the specific sample

runs. No donor sample had more than one LLOQ. The percentages of samples < LLOQ for each fluorochemical were PFOS, 0.2%; PFOA, 8%; PFHS, 48%; PFOSAA, 58%; and M570, 60%.

For all donors, the geometric mean PFOS level was 34.9 ppb (95% CI, 33.3–36.5). The range of PFOS values was < LLOQ (4.3 ppb) to 1656.0 ppb. Serum samples from male subjects had significantly ($p < 0.05$) higher geometric means for PFOS than for serum samples from female subjects (Table 1). Serum

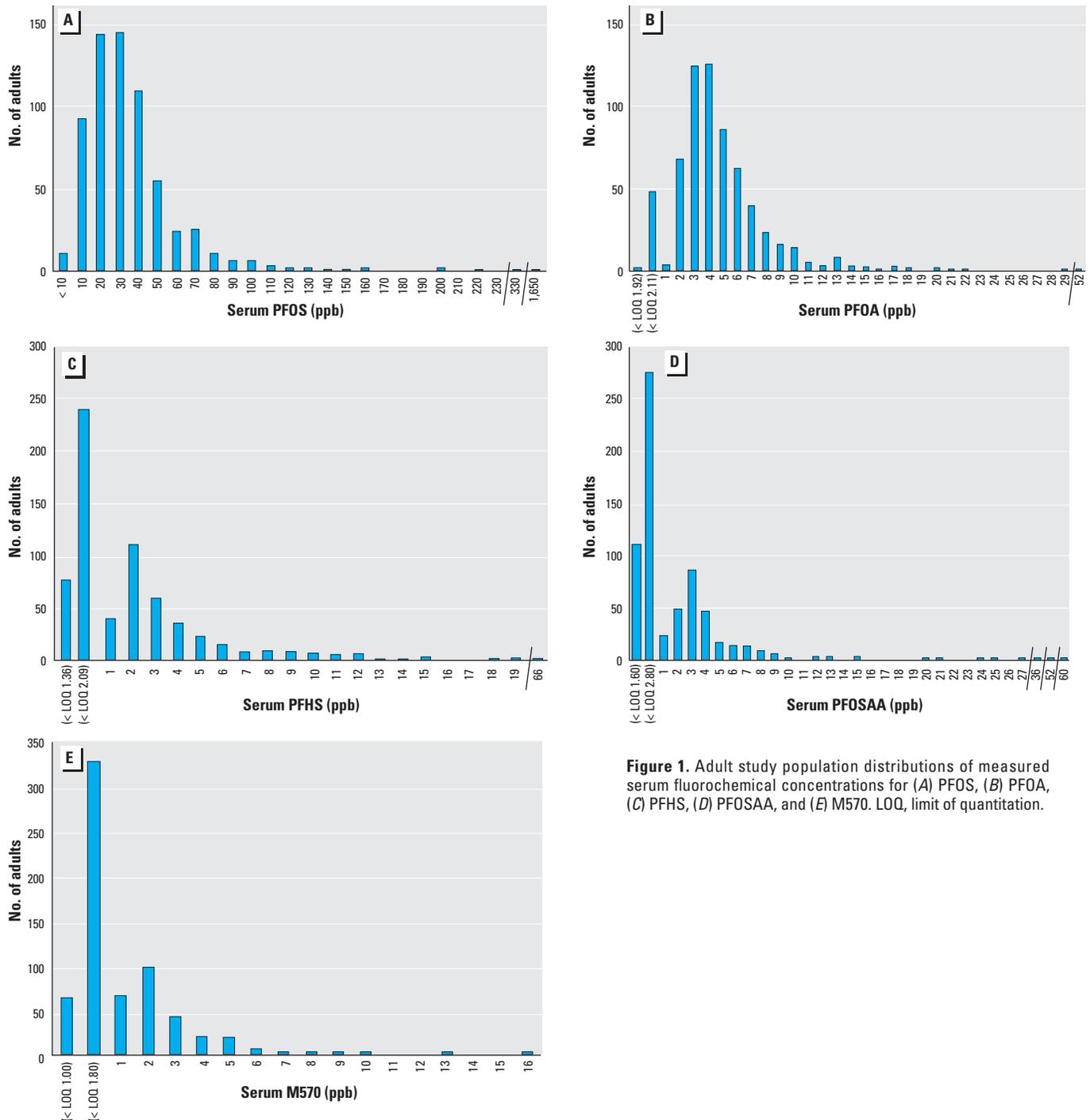


Figure 1. Adult study population distributions of measured serum fluorochemical concentrations for (A) PFOS, (B) PFOA, (C) PFHS, (D) PFOSAA, and (E) M570. LLOQ, limit of quantitation.

samples from males also had significantly higher serum levels of PFOA and PFHS compared with serum samples from females, although the mean levels for both sexes were approximately one order of magnitude lower than that of PFOS. The overall geometric mean for the calculated TOF was 31.7 ppb (95% CI, 30.4–33.0; data not shown). The calculated TOF values ranged from 5.7 ppb to 1083.2 ppb.

Age was not an important predictor of adult serum fluorochemical concentrations (Figure 2). Instances of many outliers (e.g., M570 concentrations in males 40–49 and 60–69 years of age) occurred as a result of a large percentage of values < LLOQ that were within the 1.5 × interquartile range.

The unadjusted (for age and sex) serum PFOS concentrations were higher in Charlotte compared with the other locations as a consequence of a higher interquartile range (Table 2). Table 3 shows the results from the statistical bootstrap analysis that calculated mean serum fluorochemical values for each of the six locations adjusted for 10-year age intervals, sex, and their interaction terms. There was a 10-ppb difference in the adjusted mean serum PFOS concentration between the highest (Charlotte) and lowest (Boston) locations. The mean values for the other fluorochemicals were comparable between locations. Because PFOS is the primary contributor to the calculated TOF, the bootstrap analysis findings for TOF mirrored those of PFOS.

Scatter plots (log scale) between the five fluorochemicals are displayed in Figure 3. PFOS and PFOA were highly correlated

($r = 0.63$). PFOS had a lower correlation with PFOSAA ($r = 0.42$) and lower yet with M570 ($r = 0.20$). The correlation between PFOSAA and M570 was weak ($r = 0.12$). The remaining scatter plots display the correlations between PFOS and PFHS ($r = 0.38$) and PFOA and PFHS ($r = 0.32$).

Both PFOSAA and M570, adjusted for age and sex, were significant predictors of PFOS (Table 4). PFOSAA was the more significant of the two variables (PFOSAA t -value = 14.5; M570 t -value = 4.6). Controlling for age and sex, PFOA and PFHS were also associated with PFOS.

Table 5 presents the results from bootstrap analyses conducted to provide upper tolerance limits. The upper tolerance limits represent the concentration of each fluorochemical below which the stated proportion of the population is expected to be found. The biased corrected estimates for the 90th, 95th, and 99th percentile tolerance limits of the five serum fluorochemicals and TOF along with the upper limit (bound) from the 95% CI are shown in Table 5.

Discussion

The findings from this analysis of serum PFOS concentrations in 645 adult blood donors are consistent with previous, albeit sparse, human data (3M Company 2003; Hansen et al. 2001; Olsen et al. 2003b). Serum samples obtained in the United States during the late 1990s showed mean PFOS concentrations of 30 ppb in 18 pooled blood banks, 44 ppb from a pooled commercial sample of 500 donors, 33 ppb from a different

pooled commercial sample of 200 donors, and 28 ppb in 65 commercial individual human sera samples (3M Company 2003; Hansen et al. 2001). These findings were also comparable to a limited number of serum samples from Europe that were determined to have mean serum PFOS concentrations at 17 ppb in five pooled samples from a blood bank in Belgium, 53 ppb in six pooled samples from the Netherlands, and 37 ppb from six pooled blood samples from Germany (3M Company, 2003). Mean liver and serum PFOS concentrations of 20.8 ng/g (ppb) and 18.2 ng/mL (ppb), respectively, were reported among 23 human donors with paired samples (Olsen et al. 2003b). The mean calculated TOF of 31.7 ppb in the present study is also consistent with the low-parts-per-billion TOF measurements of general population samples that have been reported since the 1960s (Belisle 1981; Singer and Ophaug 1979; Taves 1968; Taves et al. 1976).

Compared with these previous assessments, in the present study we analyzed a sufficient sample size that facilitated the characterization of the serum fluorochemical distribution by age and sex. This included the calculation of estimates of upper tolerance limits and their upper bounds. The highest serum PFOS measurement (confirmed by reassay of the sample) was 1,656 ppb. This PFOS sample approximated the average serum PFOS levels observed for POSF-related production workers (Olsen et al. 1999, 2003a); however, there is no POSF-related production plant in the Portland area. Because donor samples were anonymous, it is not possible to

Table 1. Measures of central tendency of serum fluorochemical concentrations (ppb) for American Red Cross blood donors ($n = 645$) by sex.

	PFOS	PFOA	PFHS	PFOSAA	M570
All ($n = 645$)					
Range	< LLOQ (4.3)–1656.0	< LLOQ (1.9)–52.3	< LLOQ (1.4)–66.3	< LLOQ (1.6)–60.1	< LLOQ (1.0)–16.4
IQR	24.7–48.5	3.4–6.6	< LLOQ (2.1)–3.4	< LLOQ (2.8)–3.4	< LLOQ (1.8)–2.2
< LLOQ (number)	< 4.3 (1)	< 1.9 (2)	< 1.4 (72)	< 1.6 (101)	< 1.0 (63)
		< 2.1 (48)	< 2.1 (235)	< 2.8 (271)	< 1.8 (326)
Cumulative 90%	70.7	9.4	6.3	5.2	3.8
Median	35.8	4.7	1.5	< LLOQ (2.8)	< LLOQ (1.8)
Geometric mean	34.9	4.6	1.9	2.0	1.3
95% CI geometric mean	33.3–36.5	4.3–4.8	1.8–2.0	1.9–2.1	1.3–1.4
Males ($n = 332$)					
Range	< LLOQ (4.3)–1656.0	< LLOQ (1.9)–29.0	< LLOQ (1.4)–66.3	< LLOQ (1.6)–60.1	< LLOQ (1.0)–16.4
IQR	28.3–49.7	3.6–7.0	< LLOQ (2.1)–3.8	< LLOQ (2.8)–3.3	< LLOQ (1.8)–2.2
< LLOQ (number)		< 2.1 (19)	< 1.4 (30)	< 1.6 (58)	< 1.0 (36)
			< 2.1 (104)	< 2.8 (146)	< 1.8 (163)
Cumulative 90%	72.6	10.1	7.9	4.7	3.5
Median	37.4	4.9	2.1	< LLOQ (2.8)	< LLOQ (1.8)
Geometric mean	37.8	4.9	2.2	1.9	1.3
95% CI geometric mean	35.5–40.3	4.6–5.3	2.0–2.4	1.8–2.1	1.2–1.4
Females ($n = 313$)					
Range	6.0–226.0	< LLOQ (2.1)–52.3	< LLOQ (1.4)–15.3	< LLOQ (1.6)–27.6	< LLOQ (1.0)–10.6
IQR	22.0–45.8	3.1–6.2	< LLOQ (2.1)–2.8	< LLOQ (2.8)–3.6	< LLOQ (1.8)–2.2
< LLOQ (number)		< 1.9 (2)	< 1.4 (42)	< 1.6 (43)	< 1.0 (27)
		< 2.1 (29)	< 2.1 (131)	< 2.8 (125)	< 1.8 (163)
Cumulative 90%	69.7	8.4	5.0	6.1	4.0
Median	31.3	4.4	< LLOQ (2.1)	< LLOQ (2.8)	< LLOQ (1.8)
Geometric mean	32.1	4.2	1.6	2.1	1.3
95% CI geometric mean	30.0–34.3	3.9–4.5	1.5–1.8	2.0–2.3	1.2–1.4

determine anything else about this individual besides sex (male), age (67 years), and location of the blood bank (Portland). The next highest donor level for PFOS was considerably lower at 329 ppb (also a male, 62 years of age, from the Portland blood bank location), and the next eight highest serum PFOS values (range, 139–226 ppb) were measured in four females and four males from the Charlotte

($n = 4$), Hagerstown ($n = 2$), Los Angeles ($n = 1$), and Minneapolis–St. Paul ($n = 1$) blood bank centers.

There was a relatively narrow range of geometric mean serum concentrations for the six locations studied. Because no exposure data were available, any explanation of the variation of serum concentrations once adjusted for age and sex, albeit small, between these locations

would only be speculation. As discussed above, the pathways leading to the presence of PFOS in human blood are not well characterized but likely involve environmental exposure (3M Company 2003; Hansen et al. 2002; Martin et al. 2002; OECD 2002) to PFOS or to precursor molecules and residual levels of PFOS or PFOS precursors in industrial and commercial products (3M Company 2003).

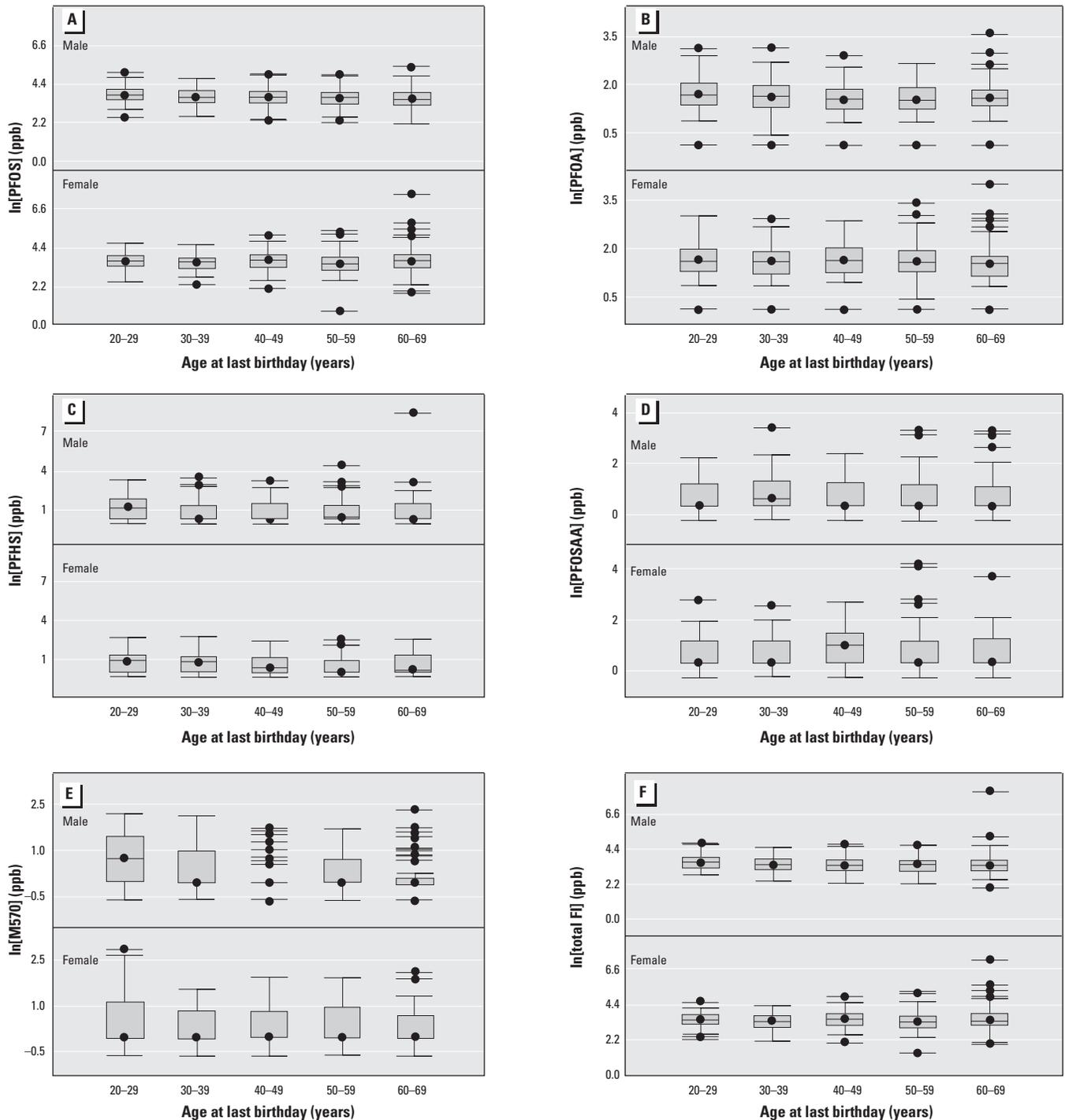


Figure 2. Box and whisker plots of serum fluorochemical concentrations by age and sex for (A) PFOS, (B) PFOA, (C) PFHS, (D) PFOSAA, (E) M570, and (F) total fluorochemicals. The boxes indicate the interquartile ranges of the natural log distributions; the circle within each box is the mean; the whiskers extend to the last observation within 1.5 times the interquartile range; and the circles outside the whiskers represent observations outside the 1.5 × interquartile range.

Our findings showed a correlation between PFOS and PFOA. PFOS has been measured in both human populations and wildlife, including marine mammals and piscivorous birds (3M Company 2003; Giesy and Kannan 2001; Hansen et al. 2001; Kannan et al. 2001a, 2001b, 2002a, 2002b). Serum PFOA concentrations, however, have been consistently quantified (i.e., measured above the LLOQs) primarily in humans. This association between PFOS and PFOA is of significant interest because PFOA cannot convert directly from PFOS (or vice versa). Whether this statistical association is due to the presence of PFOA as a by-product in POSF-related production or to other nonrelated environmental or consumer products (e.g., higher chain telomers) remains to be determined. Another unanswered question is whether perfluorooctanesulfonamides can metabolize in humans to PFOA.

PFOS was also correlated with two fluorochemicals, PFOSAA and M570. These analytes may come from oxidation of compounds used in consumer products involving paper/packaging and carpet/textile/upholstery protectants, respectively.

As with any interpretation of data obtained from a study population, questions arise regarding how well the data represent the source population and the ability to generalize from the information collected. We believe the donor selection process used in this study likely resulted in a reasonable representation of the overall donor population that was providing blood at the time when these donors were sampled. Donors were not specifically informed of the fluorochemicals assayed, nor was there any linkage between the sample collected and personal identifiers. No information was obtained about past exposure histories to POSF-related chemistries and materials or the other fluorochemicals that were analyzed. Unlike other biomonitoring efforts of environ-

mental chemicals [Centers for Disease Control and Prevention (CDC) 2003], it was not the intent of this study design to be representative of the diverse U.S. general adult population. American Red Cross blood donors are a self-selected group from the U.S. population (Allen and Butler 1993; Andaleeb and Basu 1995; Burnett 1982; Chiavetta et al. 2000; Leibrecht et al. 1976; Oswalt 1977; Wood et al. 2001). Motivations to donate blood may include altruism, desire for personal credit, low self-esteem, and social pressure. Donors in this study did not include children or the elderly. Future biomonitoring efforts by the National Center for Environmental Health at the CDC may analyze for PFOS and other fluorochemicals that will allow for a representative sample analysis of the U.S. civilian population (Calafat A. Personal communication).

A large body of toxicology and epidemiology data is available for review regarding PFOS (3M Company 2003; OECD 2002). Briefly, PFOS concentrates primarily in the liver and, to a lesser extent, in the plasma of rats (Johnson et al. 1979). There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion (Johnson et al. 1984). Studies in rodents and primates suggest toxicity varies with cumulative dose of PFOS (Butenhoff et al. 2002b; Butenhoff and Seacat 2001; Grasty et al. 2002; Lau et al. 2003; Seacat et al. 2002b, 2002c; Thibodeaux et al. 2003; Thomford et al. 2002). Lowered serum total cholesterol appeared to be a consistent early finding, with cumulative toxicity resulting in metabolic wasting and ultimately death in laboratory animals exposed to high doses. In cynomolgus monkeys, serum cholesterol levels began to decline above 100 ppm serum PFOS concentrations (Seacat et al. 2002c). The serum half-life of elimination approximated 200 days at the end of dosing (180 days, 0.75 mg/kg/day). An interim analysis estimated the half-life of serum

elimination for PFOS in humans to be approximately 9 years (standard deviation = 6) based on nine POSF-related production workers (Burriss et al. 2002). A 2-year feeding study of PFOS in rats produced a modest liver tumor response in the high-dose group (20 ppm PFOS in feed) (Seacat et al. 2002a). A 2-year feeding study of N-EtFOSE provided comparable findings (Thomford et al. 2002). PFOS and N-EtFOSE are not genotoxic in standard tests (3M Company 2003). The mechanisms of toxicity are not fully understood but may include effects on fatty acid transport and metabolism, membrane function, and/or mitochondrial bioenergetics (Berthiaume and Wallace 2002; Hu et al. 2002; Luebker et al. 2002; O'Brien and Wallace 2002; Starkov and Wallace 2002). PFOS caused modest peroxisome proliferation in rats (Berthiaume and Wallace 2002; Seacat et al. 2002b). Medical surveillance of POSF production employees has not been associated with adverse clinical chemistry or hematology results (Olsen et al. 1999, 2003a). A retrospective cohort mortality study of this production workforce reported an excess of bladder cancer based on three deaths compared with 0.2 expected (Alexander et al. 2003). It was not determined whether this association was PFOS-related or possibly due to other occupational exposures or nonoccupational exposures. Whether this is a biologically plausible association with PFOS is questionable, as PFOS did not produce bladder or other urinary tract toxicity in rat or primate chronic or subchronic studies (Seacat et al. 2002a, 2002b, 2002c). PFOS is not likely to be insoluble in human urine (solubility, 305 µg/mL at 23–24°C) at the serum concentrations present in these workers (Ellefson 2001). In a review article, Cohen (1998) reported that all of the chemical agents listed as known bladder cancer agents were genotoxic and had metabolites that were genotoxic or precipitated in the urine.

Table 2. Measures of central tendency of serum PFOS concentrations (ppb) by the six American Red Cross blood bank locations.

	Los Angeles (n = 125)	Portland (n = 107)	Minneapolis–St. Paul (n = 100)	Charlotte (n = 96)	Hagerstown (n = 108)	Boston (n = 109)
Range	6.6–205.0	6.0–1656.0	7.7–207.0	19.3–166.0	7.6–226.0	< LLOQ (4.3)–87.2
IQR	29.5–53.7	17.2–37.7	23.9–43.3	36.3–70.9	24.4–48.1	20.8–39.1
90th percentile	70.1	49.4	71.7	105.3	69.8	48.7
Median	42.2	26.0	31.7	48.9	35.7	29.5
Geometric mean	40.4	27.0	33.1	51.5	35.3	28.0
95% CI geometric mean	37.0–44.0	23.5–31.1	29.8–36.7	46.8–56.8	31.8–39.2	25.4–31.0

Table 3. Age- and sex-adjusted mean and 95% CIs of serum fluorochemical concentration (ppb) for the six American Red Cross blood bank locations.

	PFOS Mean (95% CI)	PFOA Mean (95% CI)	PFHS Mean (95% CI)	PFOSAA Mean (95% CI)	M570 Mean (95% CI)	TOF Mean (95% CI)
Los Angeles	35.0 (33.4–36.5)	4.6 (4.4–4.8)	1.9 (1.8–2.0)	2.0 (1.9–2.2)	1.3 (1.3–1.4)	30.2 (29.0–31.3)
Portland	32.8 (30.5–34.2)	4.3 (4.0–4.4)	1.8 (1.7–2.0)	2.1 (2.0–2.3)	1.3 (1.2–1.4)	28.5 (26.7–30.4)
Minneapolis–St. Paul	34.8 (31.9–36.3)	4.5 (4.1–4.7)	1.8 (1.6–2.0)	1.8 (1.7–2.1)	1.3 (1.1–1.4)	29.7 (27.4–32.1)
Charlotte	39.0 (36.2–40.7)	5.0 (4.6–5.1)	2.2 (2.0–2.4)	2.1 (1.9–2.4)	1.4 (1.3–1.5)	33.4 (31.3–35.7)
Hagerstown	34.9 (32.8–36.5)	4.5 (4.2–4.6)	1.9 (1.8–2.1)	1.9 (1.8–2.1)	1.3 (1.2–1.4)	30.1 (28.4–31.9)
Boston	29.0 (26.0–30.3)	5.3 (4.6–5.6)	1.9 (1.7–2.3)	1.6 (1.4–1.8)	1.3 (1.2–1.5)	26.4 (23.9–29.2)

Given the consistency of the data analyzed, we hypothesize that the average serum PFOS concentrations in nonoccupational adult populations may range from 30 to 40 ppb, with 95% of a population's serum PFOS concentrations below 100 ppb. After consideration of serum and liver PFOS concentrations associated with no-observed-adverse-effect levels (NOAELs) and those associated with benchmark dose values from various toxicology studies, protective values [i.e., benchmark internal concentrations (BMICs)] were chosen as "points of departure" for risk characterization (3M Company 2003). For serum PFOS comparisons, the lower 95% confidence limit of the BMIC (5% response), 31 ppm, was chosen based on rat pup weight

gain during lactation (3M Company 2003). Although rat pup weight gain was considered the most sensitive end point, comparisons were also made for other end points. For liver toxicity, a serum PFOS concentration of 44 ppm was associated with the NOAEL (3M Company 2003; Seacat et al. 2002b). For liver tumors in male and female rats, the lower 95% confidence limit of the BMIC (10% response), 62 ppm, was calculated (3M Company 2003). Therefore, for these points of departure, a human serum concentration of 100 ppb (the upper bound estimate of the 95th percentile observed in the present study) was associated with margins of exposure of 310, 440, and 620, respectively (3M Company 2003). When interpreting the significance of these margins of

exposure, it should be noted that they are based on the use of an internal dose (concentration) of a compound that is not metabolized and poorly eliminated. These facts reduce the uncertainty in considering intraspecies or interspecies response variability based on either toxicokinetic or toxicodynamic factors.

PFOA was the other fluorochemical assayed in the present study for which there are considerable toxicology and epidemiology data (Kennedy et al. In press). Sex and species differences exist in the elimination of PFOA. PFOA is rapidly cleared (hours) in female rats compared with clearance in approximately 1 week in male rats (DuPont Haskell Laboratory 1982; Hanhijarvi and Ylisen 1988; Johnson and Ober 1980). In addition

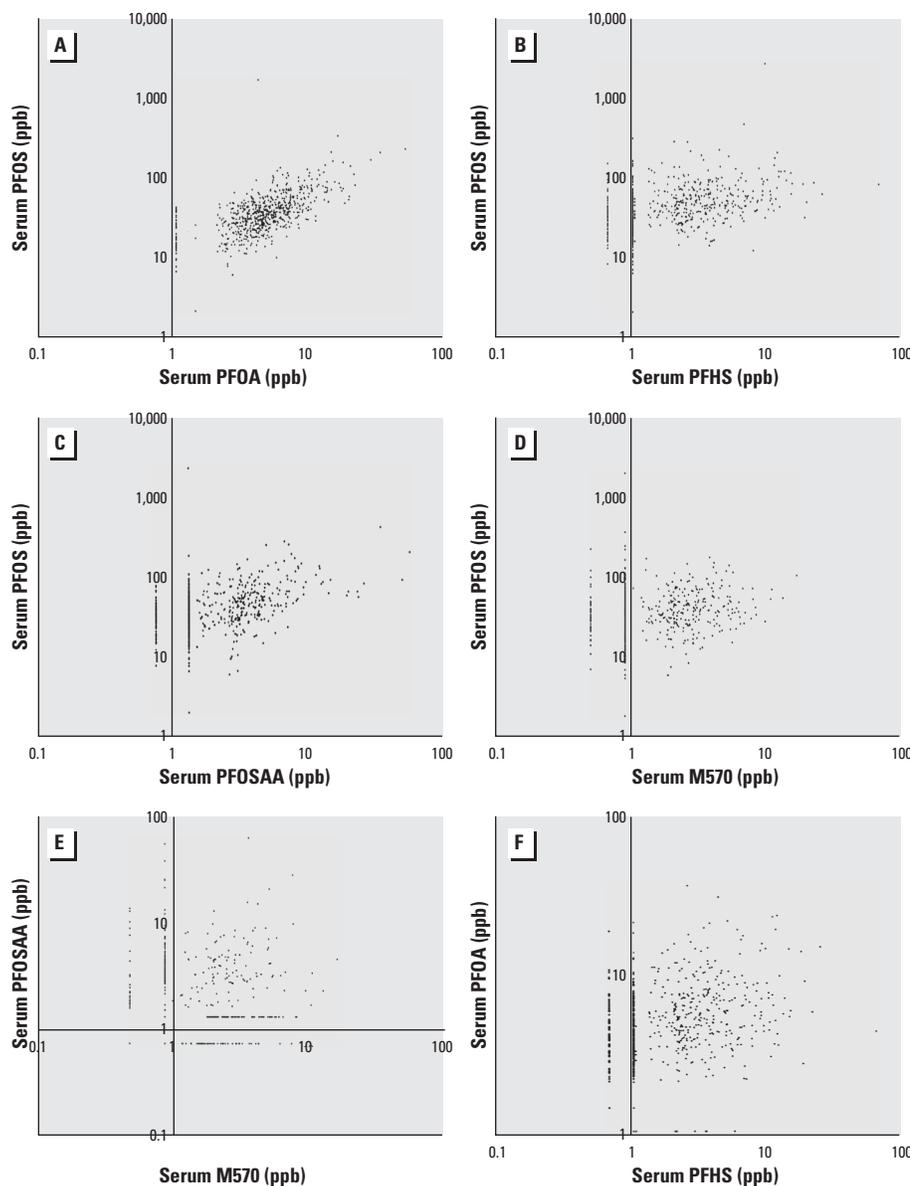


Figure 3. Scatter plots (log scale) of associations between serum fluorochemical concentrations for (A) PFOS and PFOA, (B) PFOS and PFHS, (C) PFOS and PFOSAA, (D) PFOS and M570, (E) PFOSAA and M570, and (F) PFOA and PFHS.

Table 4. Relationship between PFOS concentration and PFOSAA and M570 (Model 1), PFOA (Model 2), or PFHS (Model 3) adjusted for age and sex.

Model	Coefficient	95% CI
Model 1 ^a		
Intercept	2.323	2.167–2.481
PFOSAA	0.544	0.473–0.619
M570	0.288	0.187–0.389
Age	-0.011	-0.039–0.020
Sex	0.198	0.116–0.282
Model 2 ^b		
Intercept	2.575	2.412–2.723
PFOA	0.591	0.527–0.655
Age	-0.010	-0.010–0.016
Sex	0.074	0.005–0.143
Model 3 ^c		
Intercept	3.252	3.073–3.408
PFHS	0.267	0.213–0.317
Age	-0.008	-0.030–0.029
Sex	0.087	0.017–0.181

^a $\ln[\text{PFOS}] = \ln([\text{PFOSAA}]^a + [\text{M570}]^b) + (c \times \text{age}) + (d \times \text{sex}) + g + \epsilon$, where the serum concentration of the fluorochemical is shown in brackets and ϵ is the error term. ^b $\ln[\text{PFOS}] = \ln[\text{PFOA}]^a + (c \times \text{age}) + (d \times \text{sex}) + g + \epsilon$. ^c $\ln[\text{PFOS}] = \ln[\text{PFHS}]^a + (c \times \text{age}) + (d \times \text{sex}) + g + \epsilon$.

Table 5. Estimates (ppb) of upper tolerance limits and their upper 95% confidence limits for five serum fluorochemicals and a calculated TOF value.

Fluorochemical	Upper tolerance limit	Estimate	Upper 95% confidence limit
PFOS	90%	70.7	74.3
	95%	88.5	100.0
	99%	157.3	207.0
PFOA	90%	9.4	10.1
	95%	12.1	13.6
	99%	19.8	25.8
PFHS	90%	6.3	7.0
	95%	9.5	10.8
	99%	17.0	22.4
PFOSAA	90%	5.3	5.9
	95%	7.6	8.5
	99%	19.4	27.6
M570	90%	3.7	4.0
	95%	5.0	5.4
	99%	8.1	10.3
TOF	90%	59.9	63.1
	95%	75.1	80.9
	99%	137.3	187.5

to urinary excretion, biliary excretion and reabsorption of PFOA occur (Johnson et al. 1984). In the primate, the terminal phase elimination half-life in serum for both sexes was approximately 1 month (Butenhoff et al. 2002a). An interim analysis estimated the half-life of serum elimination for PFOA in humans to be approximately 4 years (standard deviation, 4) (Burriss et al. 2002). The liver is a primary target organ for toxicity. In rats, administration of the ammonium salt of PFOA (APFO) resulted in peroxisome proliferation, uncoupling of mitochondrial oxidative phosphorylation, increased mitochondrial DNA copy number, and altered lipid metabolism (Berthiaume and Wallace 2002; Hauglum and Spydevold 1992; Keller et al. 1992). Rats fed a diet of 300 ppm PFOA (daily dose of 15 mg/kg/day) had increased incidences of liver, Leydig cell, and pancreas acinar cell adenomas (Biegel et al. 2001). The tumors likely occurred via nongenotoxic mechanisms. Peroxisome proliferation may account for the increase in liver tumors. Enhanced hepatic aromatase activity resulting in increased estradiol may account for the Leydig cell tumors (Biegel et al. 1995, 2001; Cook et al. 1992, 1999). The pancreas acinar cell tumors were hypothesized to be a result of a mild but sustained increase in plasma cholecystokinin levels secondary to hepatic cholestasis (Biegel et al. 2001; Obourn et al. 1997). Changes in plasma cholecystokinin have not been observed in primates or humans exposed to APFO (Butenhoff et al. 2002a; Olsen et al. 2000). Hepatic toxicity, hypolipidemia, and abnormal hormone levels have not been associated with serum PFOA concentrations in APFO production workers whose serum levels have averaged 5 ppm with a range of and 0.1–114 ppm (Gilliland and Mandel 1996; Olsen et al. 1998, 2000). A retrospective cohort mortality study of APFO production workers did not report statistically significant increased standardized mortality ratios for liver cancer, cirrhosis of the liver, or pancreas cancer, although an association was observed with prostate cancer mortality and nonspecific job duration of at least 10 years in the chemical plant (Gilliland and Mandel 1993). This association with prostate cancer mortality, however, was not observed in an updated study that used an exposure matrix specific to APFO production activities (Alexander 2001).

Correction

Table 4 was accidentally omitted from the original manuscript published online and has been added here.

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